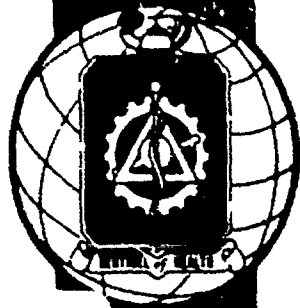
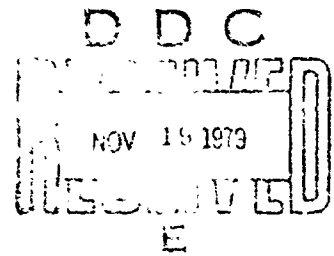


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**ABERDEEN PROVING GROUND, MD 21010**

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TOXICOLOGICAL ASSESSMENT PROGRAM STUDY NO. 75-51-0034-80  
BEHAVIORAL EFFECTS OF ACUTE  
AEROSOL EXPOSURE TO N,N-DIETHYL-META-TOLUAMIDE (M-DET)  
JANUARY - FEBRUARY 1979

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Groups of 10 male and 10 female rats were exposed for single 4-hour periods to aerosols of M-Det (an insect repellent) at concentrations estimated to be: (1) high enough to produce minimal toxic signs (4100 mg/m <sup>3</sup> ); (2) too low to produce toxic signs (2900 mg/m <sup>3</sup> ); or (3) somewhat lower (2300 mg/m <sup>3</sup> ). Concomitant controls were exposed to chamber air only.  The rats were given a battery of behavioral tests as soon as the exposure period ended. The results of the battery permitted distinctions to be made.		

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between performance at all three levels and controls for both males and females. Tests included measures of activity, endurance, balance, tactile sensitivity, post exposure learning, and memory of a task learned the day before exposure. Necropsy did not show any gross physical changes as a result of the exposures. Thus, behavioral tests were able to establish changes resulting from acute exposures to M-Det at concentrations below those at which toxic signs could be detected.

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HSE-LT/WP

SUBJECT: Toxicological Assessment Program Study No. 75-51-0034-80,  
Behavioral Effects of Acute Aerosol Exposure to N,N-Diethyl-met-  
toluamide (M-Det), January - February 1979

Executive Secretary  
Armed Forces Pest Control Board  
Forest Glen Section  
Walter Reed Army Medical Center  
Washington, DC 20012

A summary of results and conclusions of the inclosed report follows:

a. Groups of 10 male and 10 female rats were exposed for single 4-hour periods to aerosols of M-Det (an insect repellent) at concentrations estimated to be: (1) high enough to produce minimal toxic signs ( $4100 \text{ mg/m}^3$ ), (2) too low to produce toxic signs ( $2900 \text{ mg/m}^3$ ), or (3) somewhat lower ( $2300 \text{ mg/m}^3$ ). Concomitant controls were exposed to chamber air only.

b. The rats were given a battery of behavioral tests as soon as the exposure period ended. The results of the battery permitted distinctions to be made between performance at all three levels and controls for both males and females. Tests included measures of activity, endurance, balance, tactile sensitivity, post exposure learning, and memory of a task learned the day before exposure. Necropsy did not show any gross physical changes as a result of the exposures. Thus, behavioral tests were able to establish changes resulting from acute exposures to M-Det at concentrations below those at which toxic signs could be detected.

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DEPARTMENT OF THE ARMY  
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY  
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-LT/WP

TOXICOLOGICAL ASSESSMENT PROGRAM STUDY NO. 75-51-0034-80  
BEHAVIORAL EFFECTS OF ACUTE  
AEROSOL EXPOSURE TO N,N-DIETHYL-META-TOLUAMIDE (M-DET)\*†  
JANUARY - FEBRUARY 1979

1. AUTHORITY.

a. Memorandum of Understanding between the US Army Environmental Hygiene Agency; the US Army Health Services Command; the Department of the Army, Office of The Surgeon General; the Armed Forces Pest Control Board; and the US Department of Agriculture, Agricultural Research, Science and Education Administration, titled: Coordination of Biological and Toxicological Testing of Pesticides, effective 23 January 1979.

b. Letter, AFPCB, Armed Forces Pest Control Board, 17 March 1977, subject: Reregistration Data for N,N-Diethyltoluamide Repellent.

2. REFERENCES.

a. Report, USAEHA-LT, this Agency, Study No. 51-051-73/75, Development of An Efficient Test System for Assessing Behavioral Effects of Exposure to Chemical Compounds, November 1972 - November 1973.

b. Report, HSE-LT/WP, this Agency, Study No. 51-051-73/76, Behavioral and Biochemical Effects of Malathion, October 1975 - April 1976.

c. Report, HSE-LT/WP, this Agency, Study No. 75-51-0026-78, Preliminary Behavioral Assessment of Habituation to the Insecticide Permethrin, August - October 1978.

\* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education, and Welfare Publication No. (NIH) 78-23, revised 1978.

† The experiments reported herein were performed in animal facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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3. PURPOSE. To determine whether a battery of behavioral measures can detect changes in rats exposed to a single 4-hour aerosol of M-Det at concentrations below those producing minimal toxic signs.

4. BACKGROUND. Studies were conducted to determine the lethal aerosol concentration of M-Det for 50 percent of the male and female rats used as subjects (LC<sub>50</sub>). The dose response curve was found to be very steep. The LC<sub>50</sub> for the males was 6,000 mg/m<sup>3</sup> (CL = 5,000-7,400) with a slope of 9.44 (SE = 3.63). The LC<sub>50</sub> for the females was 5,860 mg/m<sup>3</sup> (CL = 4,800-7,200) with a slope of 10.99 (SE = 3.93). Because of the slight difference between effect and no toxic signs concentrations, it seemed likely that effects were occurring at lower concentrations but were not easily observable. Behavioral tests were utilized in the attempt to determine whether lower concentrations than those producing overt signs were affecting the rats.

5. PROCEDURE. Previous studies of M-Det have not addressed themselves to its behavioral effects (Ambrose, 1959,\* Ambrose and Yost, 1965,† and Rutledge et al, 1978‡) so a battery of measures was applied to three groups of rats exposed to M-Det aerosols and one chamber control group. The highest of the three concentrations used in the study was the minimal concentration previously found to produce toxic signs.

a. Animals. Forty male and forty female Sprague-Dawley albino rats were obtained from the US Army Environmental Hygiene Agency (USA-EHA) colony at a mean weight of 151 grams (+26 g) for the males and 131 grams (+15 g) for the females. They were housed in groups of five and had free access to food and water except during inhalation exposures. The food was Formulab Chow®.

b. Test Materials. The test material used was N,N-diethyl-meta-toluidide (M-Det) with a minimum meta isomer content of 95 percent and 5 percent maximum of other isomers. The material (Lot No. 7141) was manufactured by Hardwicke Chemical Company, Elgin, SC 29045, and packaged for McLaughlin Gormley King Company, 8810 Tenth Avenue North, Minneapolis, MN 55427.

\* Ambrose, A., Pharmacologic and toxicologic studies on N,N diethyl toluidide: I. N,N-diethyl-m-toluidide. Toxicology 1:97-115, 1959.

† Ambrose, A., and Yost, D., Pharmacologic and toxicologic studies on N,N diethyltoluidide II: N,N-diethyl-O-toluidide and N,N-diethyl-P-toluidide. Toxicology and Applied Pharmacology 7:772-780, 1965.

‡ Rutledge, L. C., Soffield, R. K., and Moussa, M. A., A bibliography of diethyl toluidide. ESA Bulletin 24(4):431-439, 1978.

• Formulab Chow is a registered tradename of Ralston Purina Company, St Louis, MO 63188.

c. Exposure Procedures. Rats were individually caged and placed in a Wahmann 225-liter dynamic airflow exposure chamber. The test compound was dispersed into the chamber for 4 hours with a Collision Nebulizer purchased from BEI, Inc., Waltham, MA. The rate of airflow through the chamber, as well as temperature, was continuously monitored. The actual concentration of M-Det in the animals' breathing zone was measured four times during each exposure (1/2, 1, 2, and 3 hours into the exposure). Samples were collected for analysis by pulling chamber air through a glass fiber filter at approximately 2 liters per minute for 5 minutes. The filter contents were extracted with hexane and then analyzed by gas chromatography. A Shimadzu GC-MINI-1 gas chromatograph with a 1/8-inch stainless steel 1.5-meter column containing 10 percent SP2100 on 80/100 Supelcoport (Supelco, Inc.) was used at 200 degrees Centigrade.

d. Exposure Groups. Four groups of 10 male and 10 female rats were used. The high group received the minimal concentration at which signs were originally observed by nonbehavioral methods (a time-weighted average of 4100 mg/m<sup>3</sup>). The medium group received a concentration at which no effects or toxic signs were observed (2900 mg/m<sup>3</sup>) and the low group received the lowest concentration (2300 mg/m<sup>3</sup>). The fourth group was a chamber control which was kept in a chamber identical to that used by the exposure groups but no M-Det was introduced into the air stream. The groups are illustrated in Table 1.

TABLE 1. EXPOSURE GROUPS

Group	Explanation	Concentration approx. mg/m <sup>3</sup>	Number of Rats	
			males	females
high	minimal concentration at which toxic signs were demonstrated with nonbehavioral methods	4100	10	10
medium	highest concentration at which no effects were demonstrated with nonbehavioral methods	2900	10	10
low	concentration sufficiently below the medium group so that no effects might be demonstrated with behavioral methods	2300	10	10
control	normal chamber air control - placed in chamber for same length of time as exposed groups	0	10	10

e. Toxic Signs. Fifteen commonly used toxic signs were looked for in each rat when they were removed from the exposure chamber. These are described in Table 2. The appearance of toxic signs and any other abnormalities were recorded for each rat and used to verify that the dose calculated produced the required effects or lack of them.

TABLE 2. TOXIC SIGNS

- 
1. Abnormal salivation.
  2. Frequent large swallowing movements and coughing.
  3. Lacrimation and eye bulb protrusion.
  4. Abnormally frequent urination.
  5. Fasciculations (twitching) - (a) local and (b) generalized.
  6. Tremors (shivering) - (a) head only and (b) whole body.
  7. Eye blink reflex.
  8. Hyperreactivity (exaggerated startle).
  9. Prostration.
  10. Seizures.
  11. Loss of balance while walking.
  12. Frequent or almost continuous conflict during active periods.
  13. Radical changes in the time of active periods.
  14. Obvious changes in the amount of overall activity.
  15. Grip strength as measured by resistance to pull from a grid.
  16. Eyes partially or entirely closed.
- 

f. Behavioral Measures. There are no data available to help predict which aspects of behavior are likely to be affected by exposure to M-Det, so a wide spectrum of tests covering many basic elements of the rat's behavioral repertoire were used. These tests have been used successfully in previous tests carried out in USAEHA's Toxicology Division (see references 2a, 2b, and 2c above). Because the time between the end of exposure to M-Det and the start of any test may have been critical, i.e., results were likely to change with time, all rats in each exposure group were run through each test at the same time. Thus, the time between the end of exposure and presentation of a test was kept as constant as possible for all rats in all groups. Detailed descriptions of all tests are provided in the Appendix. The order in which tests and retests were presented is given in Table 3. The behavioral measures used were:

- (1) Endurance - Grip Strength.
- (2) Passive Avoidance (post-exposure learning).
- (3) Quick Avoidance (memory).

- (4) Balance Beam (vestibular system and coordination).
- (5) Individual Short-Term Activity (tremors, locomotion, etc.).
- (6) Tactile Sensitivity (pain sensitivity and reactions).
- (7) Auditory Response (hearing and startle response).

TABLE 3. ORDER OF POST EXPOSURE TEST PRESENTATION

Test	Approx Minutes After End of Exposures
Toxic Signs	5
Quick Avoidance I	10
Balance Beam	15
Endurance	17
Auditory Check	20
Quick Avoidance II	22
Hot Plate I	28
Individual Activity	30
Passive Avoidance	40
Hot Plate II	50

g. Necropsy. At the conclusion of a 14-day post-exposure observation period, the surviving animals were sacrificed by decapitation and internal organs examined for gross abnormalities.

#### 6. STATISTICS.

a. Throughout the study, statistical significances between groups are beyond the 0.01 probability level whenever groups are said to be different from each other. This shows that if the test was repeated 100 times with different animals each time and without any rats being exposed, only once in the hundred times would the results for each group be as different from each other as they were when the actually reported tests were done. Thus, there

is only one chance in 100 that the two groups being compared are actually not different. Most of the results are actually different at the 0.005 level (one chance in 500 of the groups not being different). In toxicological studies, 0.05 (rather than 0.005) is usually the accepted level of significance. However, as there is frequently great variability between individual's performances in behavioral tasks, a level of 0.01 is more appropriate to further insure avoiding the mistake of concluding a real difference is present when two groups are actually only different due to random variation.

b. When the data from each rat in all four groups could be compared, a linear regression test was used to determine whether a significant dose-response relationship existed. The degree of correlation between the test results and the exposure concentrations can be evaluated for significance. In other words, an excellent dose-response correlation would be a doubling of the test scores when the concentration is doubled, with no change in variability. How close all the experimental groups come to maintaining this "linear" type of relationship, and the amount of variability in the scores is what is being evaluated.

c. The differences between the means of each group along with the variability around the means were tested with paired two-tailed student "t" tests to identify which groups were different from each other. "Two-tailed" refers to the idea that no prediction was made as to how the groups would differ (i.e., would mean "A" be higher than mean "B" rather than just different?). If such a prediction had been made, the result would be doubly significant because not only was a given level of difference correctly predicted, but also its direction. The student "t" test compares the means between two groups and uses the amount of variation around each mean to judge how likely it is that the means are different only by chance variation in the sample. This test can only be used if the two samples have similar amounts of variation. In cases where there is an extreme difference in variation or distribution of scores between the two groups, a ranking test such as the Mann-Whitney "U" is used. This test ranks the scores in the two groups from lowest to highest and evaluates the amount of overlap in ranks.

d. When group means are presented, they are always followed with the standard deviation in parenthesis. About 68 percent of the sample population is within one standard deviation above and below the mean.

e. Much of the data can only be presented in "raw" form, e.g., how many animals fell off a balance beam. These type of data does not provide scaled values for each rat so group means or other measures of central tendency and variation cannot be made and cannot be evaluated by statistical techniques beyond graphic comparison unless the experiment is repeated, thus, the experimenter and reader must decide whether an actual difference exists.

7. RESULTS. The combined results of the battery of tests showed distinct differences among the three concentration levels and the controls. The two lower concentration levels were relatively close (2900 and 2300 mg/m<sup>3</sup>) so it is not surprising that only a few tests differentiated between the two. There were major differences between which tests showed differences for the males and females. Results for the male and female members of the same group (i.e., animals receiving the same concentration) were frequently statistically different. The results of all tests are summarized in Table 4; Table 5 summarizes the study's results and depicts which tests were effective in differentiating between the various concentrations.

a. Weight. The males in all four groups were statistically similar to each other both the day before and 2 weeks after exposure. The same was true for the females. The males and females gained the same proportionate amount of weight but were significantly different from each other both before and after the exposure. The weights are presented in Table 4.

b. Toxic Signs. Each rat was examined just after removal from the chamber and the list of standard signs depicted in Table 2 was used as a guide to look for unusual behavior or other effects. The only animals showing signs were the high concentration group. Two males showed obvious tremors but no other signs were definitely present. However, the males as a group appeared somewhat quieter than normal. One of the females was prostrate, three could not walk, and two could not maintain their balance. Three of these six were shaking moderately. Of the ten females, four showed no signs and, of the ten males, eight showed no specific signs, but all rats appeared to lack energy.

c. Endurance.

(1) The ability to hold onto a horizontal rod by the front paws was different for males and females. Control group females held on longest and the high group held shortest with the medium and low groups spaced evenly between to give the best dose response curve in the study ( $r = 0.7906$ ;  $\text{sig} = 0.001$ ). Males showed a similar trend with the exception of a longer ability of the medium group to hold on than would have predicted from the female's data ( $r = -0.7035$ ,  $\text{sig} = 0.01$ ). These data are summarized in Table 4 and illustrated in Figure 1.

(2) Seven control female rats were able to hold onto the bar for the entire length of the test (30 seconds) while no low or medium and only one high group female held on for that long. Thus, controls clearly had far greater ability to continue holding onto the rod than the exposed animals.

d. Passive Avoidance. Among females, the number of seconds required to learn to stay in the safe corner and, thus, avoid any possibility of shock was significantly less (0.005) for controls than the high group with the

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TABLE 4. DATA SUMMARY (When group means are shown, they are followed by their standard deviation in parentheses)

OBSERVATION		SEX	CONTROL	CONCENTRATIONS		
				LOW	MEDIUM	HIGH
A. Toxic Signs		F	none	none	none	4 normal; 1 not walking 3 shaking; 1 prostrate; 2 losses of balance
		M	none	none	none	2 shaking; 8 normal
B. Weights (gm)	exposure day	F	137(22)	134(14)	130(13)	123(9)
		M	167(25)	164(24)	142(11)	142(19)
	weeks after exposure	F	171(20)	178(18)	173(15)	182(17)
		M	231(18)	218(30)	219(18)	223(27)
C. Endurance	No. sec held on	F	24(8)	15(8)	10(9)	5(4)
		M	26(8)	18(13)	25(11)	10(3)
	No. held on for 30 sec	F	6	0	1	0
		M	7	3	7	1
D. Passive Avoidance	No. sec after last time left safe area	F	64(27)	36(25)	49(43)	21(26)
		M	37(33)	40(32)	46(36)	36(34)
	No. shocks	F	4.4(2.3)	6.3(3.1)	6.1(5.0)	14.2(5.9)
		M	6.4(4.1)	5.7(3.5)	6.3(7.3)	3.9(3.1)
E. Quick Avoidance	training trials	F	5(7)	4(5)	6(7)	3(5)
		M	2(1)	4(5)	4(1)	6(10)
	(No. sec to leave shock box)	F	3.4(4.4)	3.2(3.1)	2.6(1.9)	12.1(17.5)
		M	1.7(0.7)	6.9(13.5)	8.1(13.4)	3.1(3.4)
	trial II (12 min after trial I)	F	10.7(14.0)	8.5(13.7)	8.1(7.8)	14.4(16.7)
		M	2.7(1.1)	4.6(7.2)	15.3(15.5)	10.8(12.9)

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OBSERVATION		SEX	CONTROL	CONCENTRATIONS		
				LOW	MEDIUM	HIGH
F. Balance	scores	F	18	14	14	35
		M	10	14	13	34
	No. did not move	F	2	5	4	2
		M	2	7	5	7
	No. walked off	F	2	1	1	2
		M	2	4	4	1
	No. fell off the beam	F	3	1	0	6
		M	1	1	1	2
	No. slips	F	2	1	8	5
		M	2	3	6	11
G. Activity	errors	F	207(53)	232(140)	239(166)	76(33)
		M	216(112)	300(109)	224(92)	212(74)
	medium & large movements	F	11(4)	11(7)	16(11)	2(4)
		M	19(7)	14(8)	18(10)	8(8)
H. Tactile Sensitivity (seconds to respond to heat)	first trial	F	14(10)	10(5)	12(8)	15(12)
		M	18(9)	12(4)	15(6)	12(8)
	Second trial	F	18(8)	8(2)	16(9)	15(11)
		M	24(8)	11(7)	13(8)	9(8)

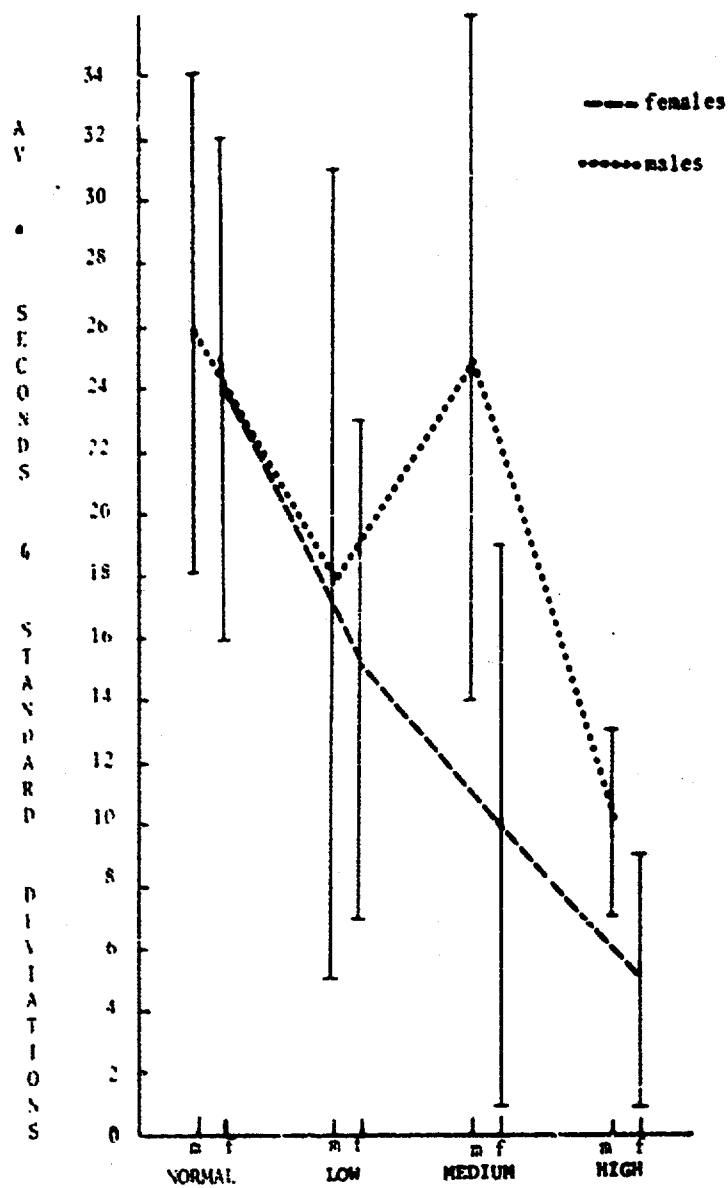


FIGURE 1. Endurance - Number of seconds held onto a horizontal rod.

medium and low groups falling in between. "T" tests showed that the high group received significantly (0.001) more shocks than the other groups. There were no differences among the male groups for either measure. The data are summarized in Table 4, Appendix, and illustrated in Figure 2.

e. Quick Avoidance.

(1) There were no significant differences among any of the groups in preexposure learning of the quick avoidance task. Among females in trial one, the high group required significantly longer to leave the shock chamber than the other groups. Their variation was also significantly greater so the "U" test was used instead of the "t" test to evaluate differences. There were no differences for trial two. The variation for the high group was 17.5 but only an average of 3.1 for the control, medium, and low groups so the differences in variation are actually important in themselves.

(2) Among males, there were no differences for trial one but trial two showed the normal and low groups to be much faster than the high and medium groups. Because the higher concentration groups had much higher variabilities (11.3) than the normal and low concentration groups (3.5), the "U" test was used and the difference was significant. The data are presented in Table 4, Appendix, the number of seconds to leave the shock box are illustrated in Figure 3, and the variability is illustrated in Figure 4.

f. Balance.

(1) Scores. The balance scores for the males showed a progression from minus 10 for the normals through plus 14 for the low and medium groups to plus 34 for the high group. The females' values were virtually identical to the males' scores except that the normals were plus 18.

(2) Movement. The number of rats who did not move showed a dose-response relationship for the females (8, 5, 4, and 2) but only the normals were different from the other three groups for the males (2 vs 7, 5, and 7). Among males the number who walked off was much higher for the normal group (8) than for the high group (1) with the low and medium groups in between (4 and 4). There were no differences for the females in this measure.

(3) The number of rats falling off the beam was higher for the high female group (6) than any of the other groups. The number of slips made while on the beam showed a dose response correlation for the males but not for the females. These data are presented in Table 4. The balance scores are illustrated in Figure 5 and the raw scores for each of the measures discussed above are depicted in Figure 6.

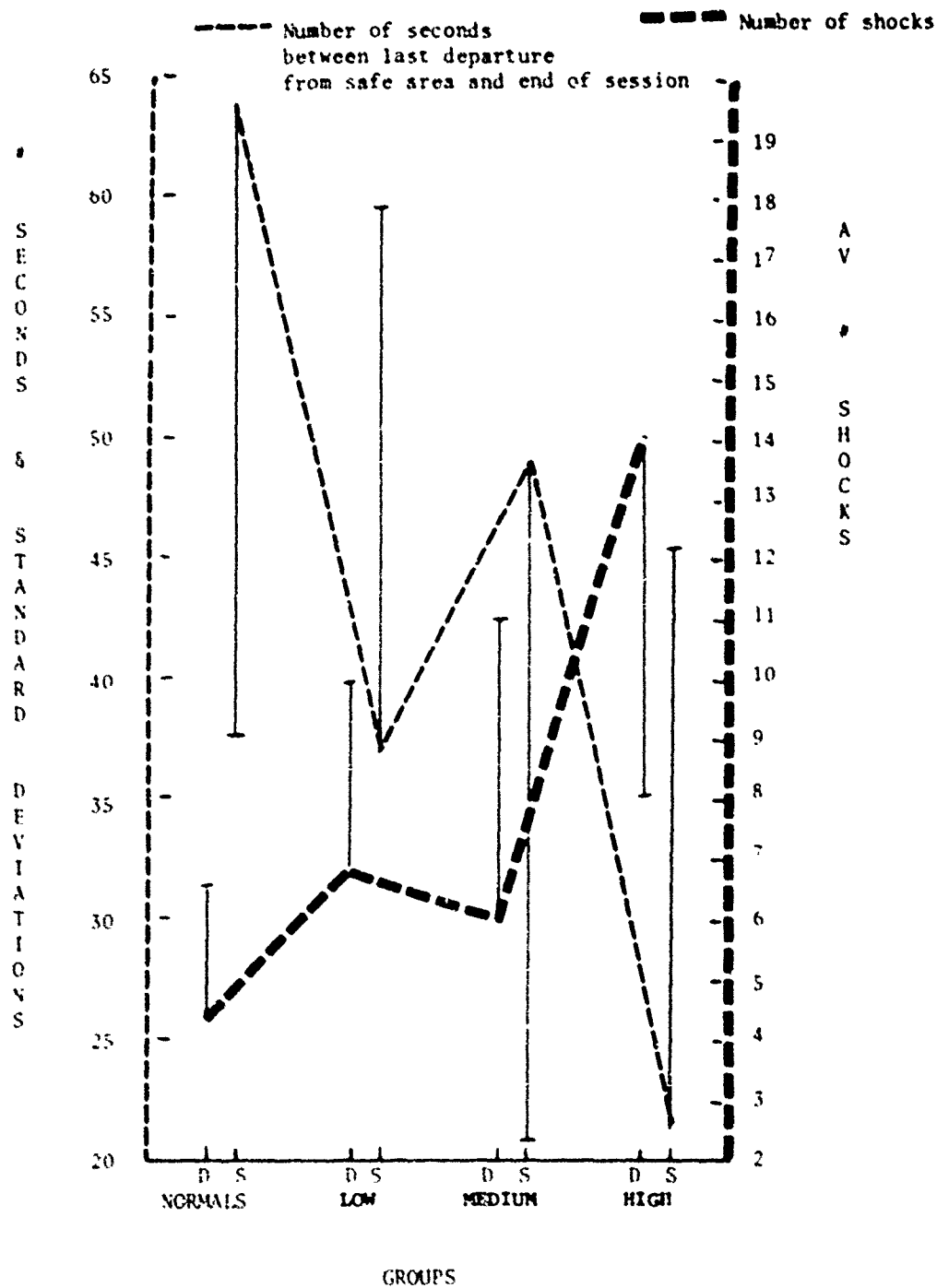


FIGURE 2. Passive Avoidance - Females.

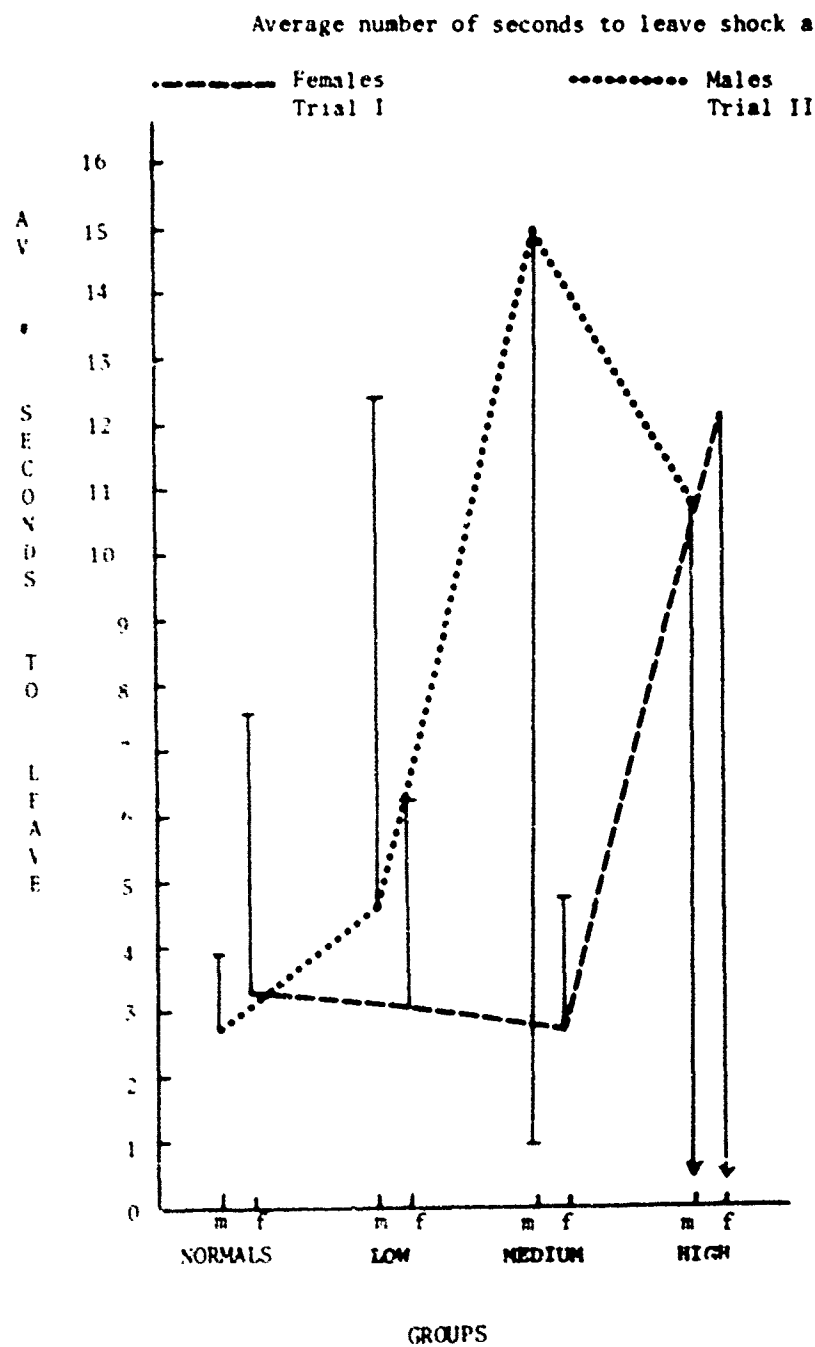


FIGURE 3. Quick Avoidance.

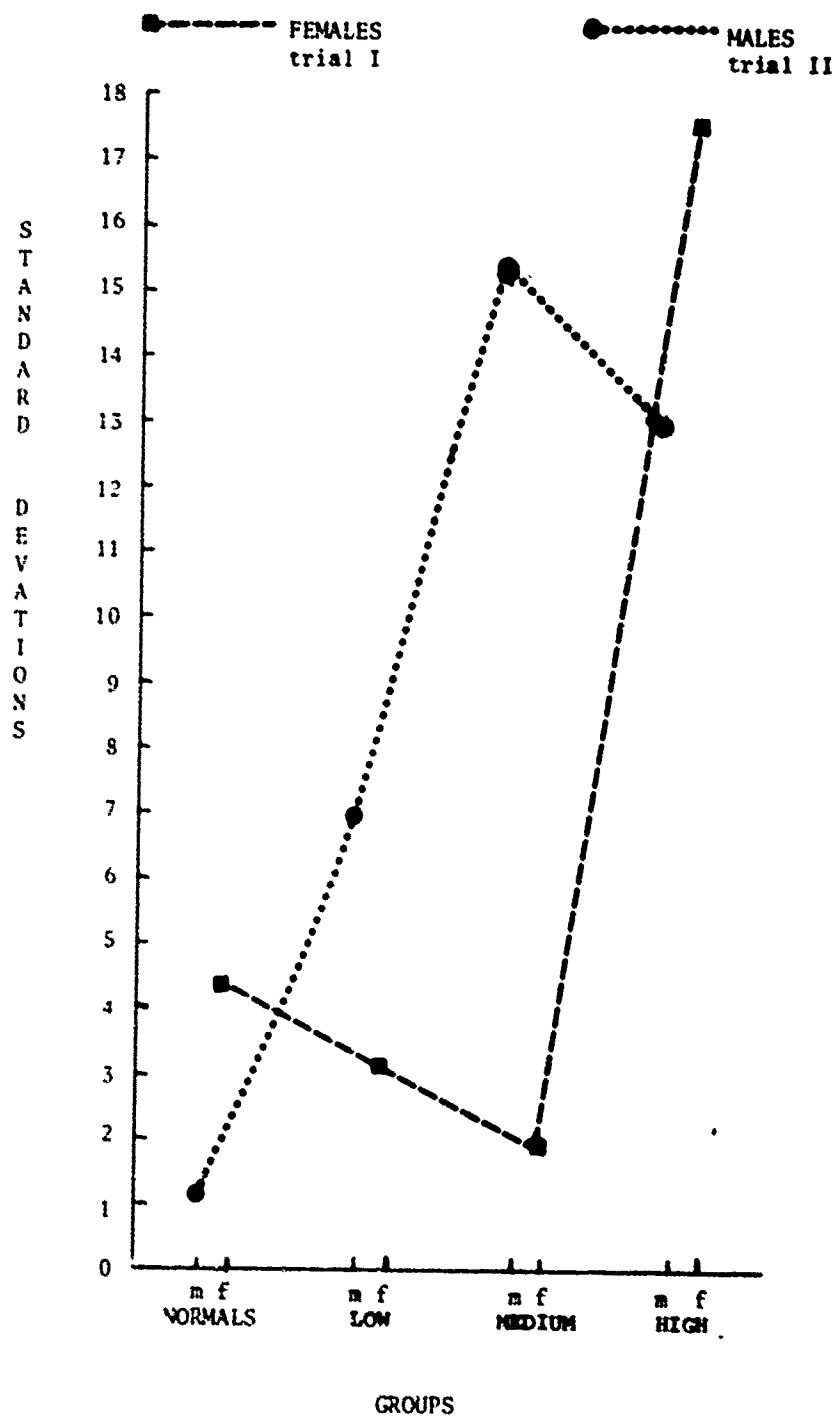


FIGURE 4. Variation in Quick Avoidance Times.

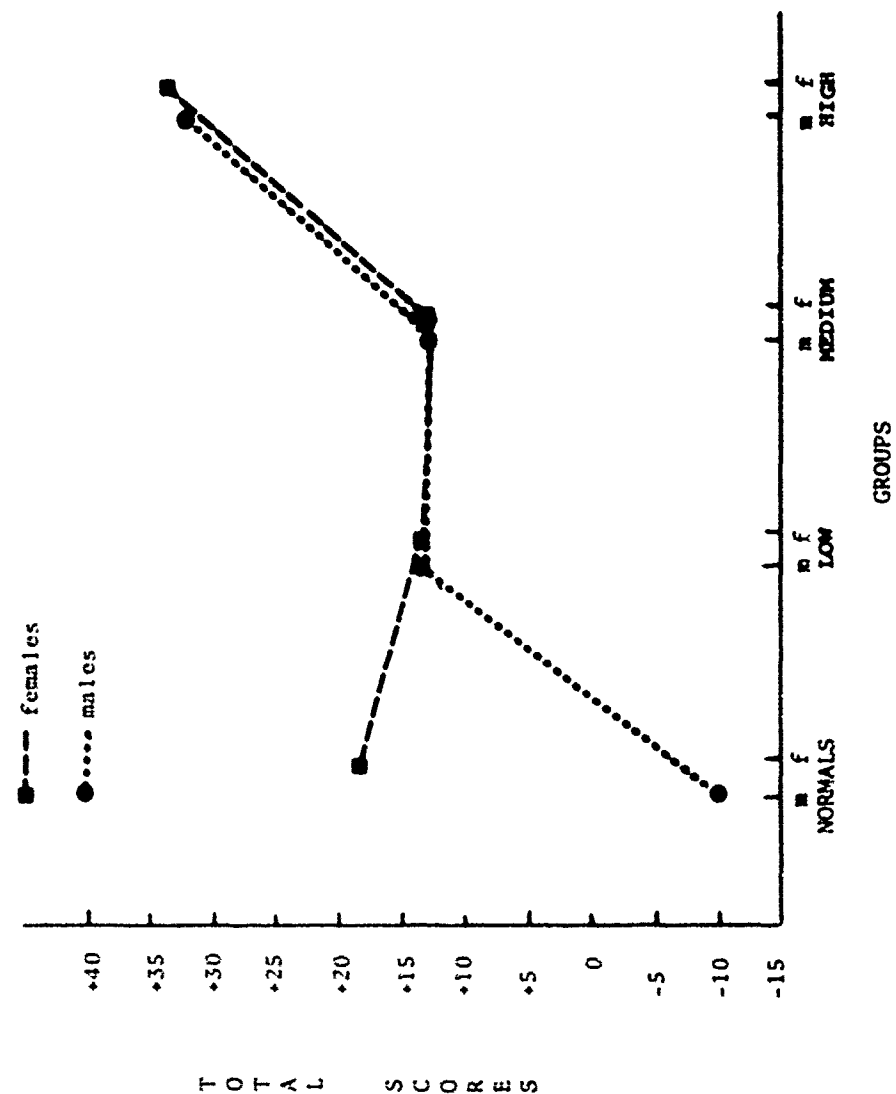
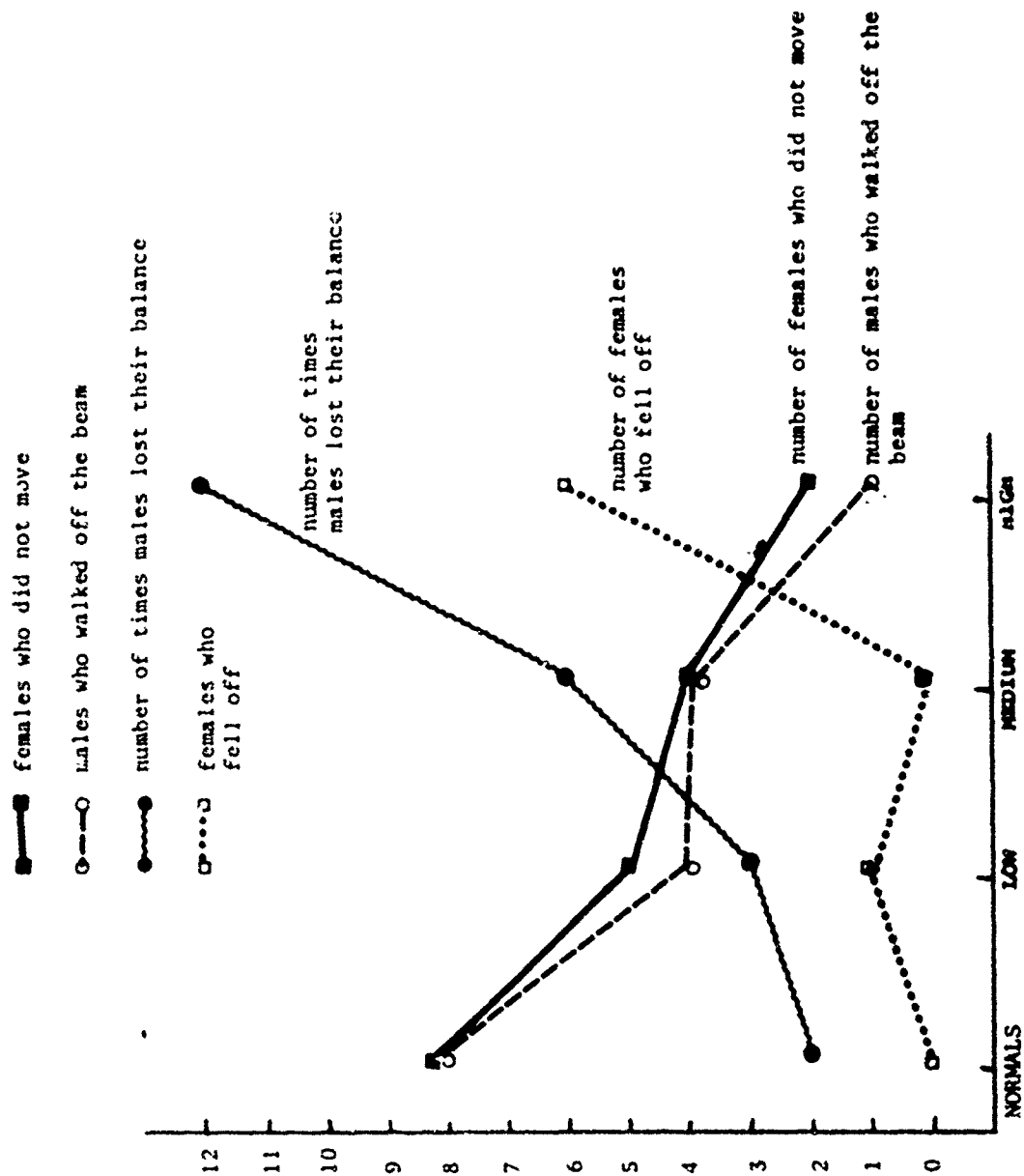


FIGURE 5. Balance Scores.



GROUPS

FIGURE 6. Balance - Raw Data.

g. Activity. The average number of small movements made by the high female group was significantly less than that of the other three female groups. The males followed a parallel pattern but the difference was not significant. The data are presented in Table 4 and illustrated in Figure 7.

h. Tactile Sensitivity. The normal male group took significantly longer to respond to the heat than the other groups in trial two. There were no other differences among either male or female groups. The data are presented in Table 4.

i. Auditory Response. There were no differences between any of the groups. Virtually all rats showed normal responses.

j. Results of the Necropsy. None of the rats showed gross abnormalities. No micropathological, chemical, or histochemical tests were performed.

## 8. DISCUSSION.

a. No one test used in this study differentiated between all groups of both sexes. However, individual tests did produce consistent, statistically significant differences between several groups. When the results of all the tests were compiled, all variables were readily differentiable from each other. Table 5 contains this compilation. The results clearly show that a combination of behavioral measures were able to differentiate between concentrations of M-Det used and that behavioral measures can demonstrate differences between groups of animals receiving concentrations below those causing any gross physical changes or toxic signs and normal animals. The auditory function test was the only member of the battery not to show differential effects among any groups. The key to the value of a multiple test screen is its ability to reliably differentiate between many doses of a compound by combining results of many tests where each test is able to show consistent differences between only several of the many doses. The compilation of results from individual consistent tests gives consistent results over a far wider range than an individual test could have provided. A single rather ineffective test repeated many times might randomly show some groups as being apparently different than others in random directions. Thus, the true value of the screen is that it can do more than any one test and do it consistently, quickly, and effectively.

b. A typical 56-gram aerosol can (e.g., Federal Stock Number 6840-864-5434 with Specification Number 0-1-503E) contains about 40 grams of M-Det at a concentration of 71 percent. A typical spray nozzle emits about 1 gram per second and it takes about 10 seconds to spray oneself with an insecticide. If this were done in a small 10-cubic meter room, the occupant would be exposed to about  $0.7 \text{ g/m}^3$  of M-Det. The lowest concentration tested was  $2.3 \text{ g/m}^3$  which is 3.3 times the likely aerosol exposure concentration and

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Average number of movements  
and standard deviation

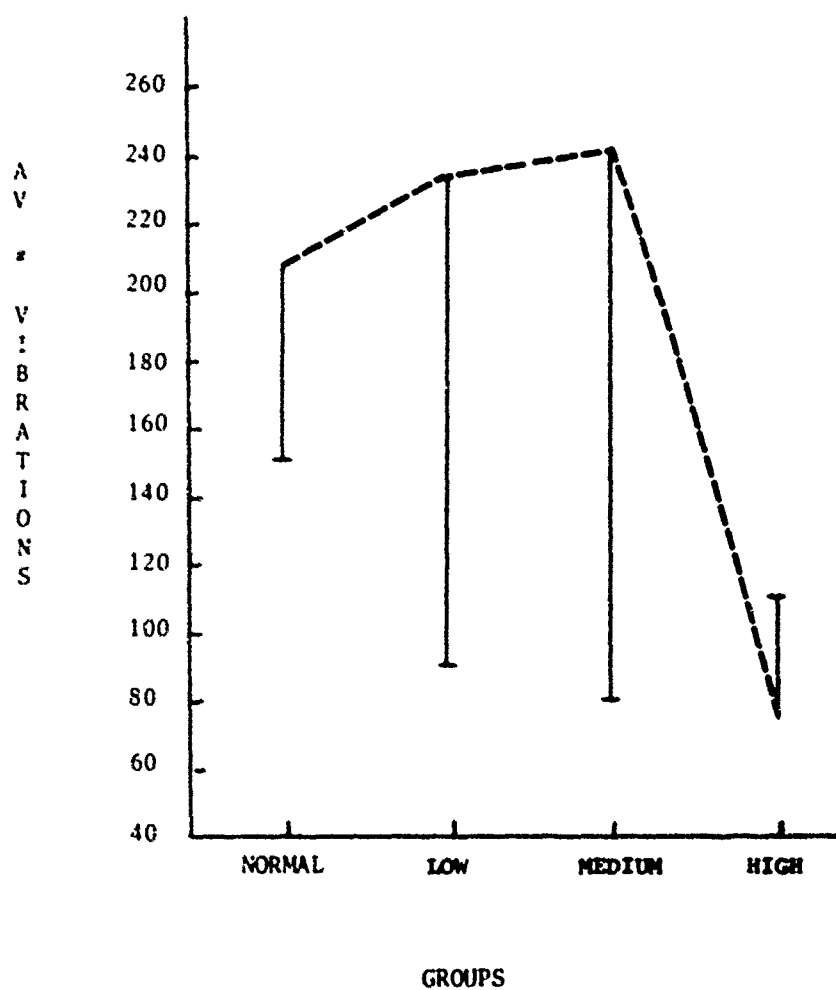


FIGURE 7. First Minute of Activity - Females.

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TABLE 5. TEST AND CONCENTRATION DIFFERENTIATION SUMMARY

Lines between concentration levels indicate significant differences detected by a test. A lack of lines indicates no difference. C = control (no M-Det), L = low, M = medium, and H = high concentration. For example, c-lmh means that the control group is different from the low, medium and high groups who are statistically similar to each other.

TEST	SEX	ACUTE
endurance	f m	c-l-m-h mh, cm-l, cm-h, l-h
passive avoidance	c-l-m-h m	clmh
quick avoidance	f m	clm-h cl-mh
balance	f m	c-l-m-h c-l-m-h
activity	f m	clm-h clmh
tactile sensitivity	f m	clmh c-lmh
DIFFERENTIAL SUMMARY	F M	C-L-M-H C-L-M-H

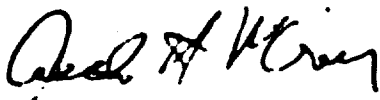
was for 400 times as long. Thus, the results of the present study should not be interpreted as indicating that exposure to normal levels of M-Det causes clinically significant behavioral changes because of the difference between realistic and experimental concentrations and durations. A 13-week study using lower concentrations has recently been completed so data on long-term, low-concentration exposure will be available shortly. Further discussion of dose-response anomalies and relationships of human function and health to changes in rodent's responses on behavioral tests will be presented in the subchronic report.

9. CONCLUSIONS AND RECOMMENDATIONS. The behavioral measures screen was able to differentiate the three exposure levels from one another and from the controls at statistically significant levels. However, far more work with lower concentrations will be needed before the biological significance of the tests can be established.



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## APPENDIX

### BEHAVIORAL TESTS USED IN EVALUATING THE EFFECTS OF ACUTE EXPOSURE TO M-DET

#### 1. GRIP STRENGTH - ENDURANCE.

a. Rationale - This test measured the rat's ability to hang from a rod to evaluate changes in strength and endurance.

b. Description - The rat was suspended from a thin rod by his front paws and the number of seconds until he let go were noted.

c. Procedure - The apparatus consisted of a 1/8-inch diameter fixed rigid rod suspended 1.5 meters above a foam pad. This was high enough so rats did not just let go but could not be harmed by a fall. After exposure, the rat was hung from the rod by his front paws and the number of seconds to release was measured. The test was terminated after 30 seconds. If the rat grabbed the bar with a rear paw or his tail during the test, the timer was stopped. The extra paw or the tail was gently removed from the rod and the timer was restarted.

#### 2. PASSIVE AVOIDANCE.

a. Rationale - This learning task measured post-exposure ability to learn a task not requiring extensive coordination, strength or mobility.

b. Description - After exposure, rats learned to avoid an intermittent, mild foot shock by finding and then remaining in the "safe" corner of a cage. The shock train was on when the corner was vacant.

c. Procedure - The apparatus consisted of a 30 x 21 cm cage with a standard grid floor. A train of 2.0 mA 1-second shocks 9 seconds apart was presented through the floor by a BRS/LVE SGS-003 shock scrambler. A photobeam crossed a rear corner of the cage. When the beam was not interrupted, the shock train was on. The rat could keep the beam interrupted and, thus, avoid shocks by identifying and remaining in the "safe" corner. The shock was the minimum intensity to promote a reaction but not sufficient to cause the rat to jump or squeak. The number of seconds the beam was interrupted and not interrupted were automatically recorded each time the beam was broken. After exposure, the rat was placed in the safe corner (nose in) and the apparatus was turned on. The test lasted 2 minutes and, as only one shock was delivered every 10 seconds, a maximum of 12 shocks could have been presented. The number of shocks presented, number of times the safe area was left, number of seconds in and out of the safe area, and the number of seconds between the last time the safe area was left and the end of the session were automatically recorded.

### 3. QUICK AVOIDANCE.

a. Rationale - This memory test measured post-exposure retention of a task learned just before exposure.

b. Description - The day before exposure, rats were trained to leave a small box to escape from an ongoing mild shock. After exposure, they were replaced in the box but the shock did not start for 20 seconds. They could avoid any shock by leaving the box within 20 seconds.

c. Procedure - The apparatus consisted of an opaque lidded rectangular start box 8 x 21 x 8 cm high with a 6 x 6 cm open doorway at the end of one side. The doorway led to a dark 20 x 24 cm safe area. The shock was presented through a standard floor grid by a BRS/LVE 1311 shock scrambler and was the minimum intensity able to promote a consistent response (2 mA). It was a maximum of 40 seconds in duration and not intense enough to cause the rat to jump or squeak. The day before exposure, rats were given four training trials, 5 minutes apart, in which they learned to escape a mild foot shock by leaving the start box. The rat was placed into the start box facing the rear. The shock and a 1000 Hz, 60 dB warning tone started when the rat interrupted a photocell beam while being introduced into the box. A timer was started by interruption of the start box's photocell beam and stopped when the rat interrupted a second beam while entering the safe area. Rats not escaping the shock on any trial were not used in the post exposure tests. After exposure, the rat was given three test trials in which the warning tone started immediately but the shock was delayed by 20 seconds. The rat could avoid being shocked by leaving the start box within 20 seconds. The test was terminated after a maximum of 40 seconds. The number of seconds to leave the start box were recorded onto paper tape automatically.

### 4. BALANCE BEAM.

a. Rationale - Motor coordination and the vestibular system were both measured by determining the rat's ability to move on a relatively narrow board.

b. Description - After exposure, rats were placed on the beam and their ability to move were objectively measured by counting four distinct behaviors including falling off and centimeters walked along the beam.

c. Procedure - The apparatus was a 2 cm wide by 2 m<sup>3</sup> long rough wood beam. One end was clamped onto a counter top to provide stability and an obvious exit from the beam. A start line was marked 45 cm from the end distal to the counter top and the beam was divided into 5 cm segments by numbered lines. The beam was 1.5 m above a foam pad so that the rat was not likely to jump off but would not be harmed by a fall. After exposure, the

rat was placed with his nose at the start line and given a maximum of 30 seconds on the beam. The rat was handled only in those rare cases in which he turned around to race the end of the beam away from the counter. Number of losses of balance, number of centimeters moved, seconds to walk off the beam, and seconds to fall or no fall were recorded. Balance scores were compiled for each group by adding the following numbers for each rat making the following moves:

- 2 : walks off.
- 1 : each loss of balance.
- 2 : does not move for 30 seconds.
- 5 : falls off.

#### 5. INDIVIDUAL SHORT-TERM ACTIVITY.

a. Rationale - The test measured exploratory activity of an individual rat placed into a novel, unobstructed environment. The amount of exploratory movement, the number of times the rat defecates, and whether or not he urinates, have been related to aggressiveness (Hinde 1966)\*. Thus, the test indirectly measured the level of aggressiveness as well as motor activity. The sensor also measured tremors which are a frequent result of exposure to chemicals.

b. Description - Individual rats were placed into a rectangular cage of a type they had never been in before. The cage was mounted on a sensor which was sensitive to slight tremors, nonambulatory motions such as grooming and standing up, as well as ambulatory movements such as walking and jumping. All three types of movement were recorded automatically while the rat explored the cage.

c. Procedure - The apparatus was a 45 x 22 x 21 cm plastic cage mounted on an activity sensor. Cases were washed with warm water after each occupant to avoid introducing variables due to leftover pheromones and other odors. The sensor counted vibrations (e.g., tremors), nonambulatory movements (e.g., grooming), and ambulatory movements (e.g., walking) separately and automatically printed out totals every 10 seconds. After exposure, a rat was placed into the cage and his activity is recorded for 2 minutes. The data from the three activity levels and the number of feces and whether or not the rat urinated were recorded.

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\* Hinde, R., Animal Behavior, McGraw-Hill, New York, 1966, p. 535-536.

6. TACTILE SENSITIVITY.

a. Rationale - The test measured the rat's responsiveness to heat. This was important not only for measuring changes in pain sensitivity but also because changes in tactile sensitivity effect sensitivity to foot shock and, perhaps, the results of the quick and passive avoidance tests.

b. Description - The rat was placed on an uncomfortably warm surface and the time to initially respond to the heat was measured.

c. Procedure - The apparatus consisted of a 20 cm diameter by 20 cm high kimax glass drum whose floor was heated to 65 degrees Centigrade (2 degrees drift per hour) by a hot plate controlled by a thermister activated regulating system. Sixty-five degrees is uncomfortably warm to the pressed hand but will not burn. A rat was dropped into the drum from 10 cm above its floor so that it landed on all four feet. The number of seconds until the rat licked a hind foot or jumped out of the drum was recorded and the rat was removed. When there was no reaction, the test was terminated after 30 seconds.

7. AUDITORY FUNCTION AND REACTIVENESS CHECK. Hyperreactivity and unresponsiveness are both common reactions to toxic insult as are radical changes in ability to see, hear, and feel. Auditory function and reactivity were checked by placing the animal alone in a quiet room and clapping behind him. The presence or absence of a visible motor response immediately following the sound was noted. The responses counted were sudden turning of the head, reorienting of the body, startle reactions (muscles suddenly jerking tense) or major rippling of the skin. The extent of reaction was rated on a scale of zero to four as follows:

- 0 - No reaction (extra trial given to be sure).
- 1 - Muscle tensing and rippling but NO reorientation of head or sudden major motor movements.
- 2 - Reorientation of head or body but no sudden jumps or squeaks.
- 3 - Jumping and/or squeaking.
- 4 - Convulsions.